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PROBABLE SYNTHESIS OF PORPHINE-LIKE SUBSTANCES DURING CHEMICAL EVOLUTION

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INTRODUCTION

It is already a well established theory that chemical evolution, i.e. evolution on the molecular level, has preceded the appearance of the first living cell on the earth. Current concepts suggest that a relatively simple primitive atmosphere, consisting of methane, ammonia, water, and also probably some molecular hydrogen under influence of ultra-violet radiation, electrical discharges and high energy radiations has been transformed into more complicated compounds (1, 2, 3). It has been generally accepted that the temperature at the time of this transformation was less than 100°C. The raw material thus produced, accumulated in pools of water that condensed, forming an organic millieu, i.e. the non-living mixture of carbon compounds which is generally considered as a prerequisite of the origin of life. Some estimates made by Urey (2) indicate that the primitive oceans might have contained as much as 10 per cent of dissolved organic compounds. The synthesis of these raw materials continued until a state of equilibrium was reached, in which the action of available energy sources produced as much of the raw material as it destroyed. In this period of time only relatively simple molecules could be formed because the more complicated and labile ones would be destroyed by the energetic ultraviolet radiation reaching the surface of the earth, unscreened by the ozone layer, which now protects the earth.

The above theories of Oparin (1) and Urey (2) have been subjected to rigid laboratory tests by Miller (4, 5), Pavlovskaya and Pasynskii (6), Abelson (7), Oro (8), Hasselstrom (9), Paschke (10), and others (11, 12). This laboratory work has produced various products of biochemical importance, such as amino acids, aliphatic acids, polyhydroxy compounds, aldehydes and urea. Subjecting these simple molecules to further action of ultraviolet radiation or mild heating, produced a variety of complex organic molecules. Such higher order reactions, for example, result in production of polypeptides from amino acids, as demonstrated by Fox (13, 14), Akabori (15), Doty (16), and others (17), in actual laboratory experiments.

In the reviewed literature, most of the authors (1, 18, 19, 20) dealing with the problem of the origin of life, are convinced that at some stage of chemical evolution, porphyrins must have developed. However, until recently there was no laboratory evidence that porphyrins or porphine-like substances could be synthesized from precursors which were available during the period of chemical evolution on earth.

This paper presents observations having direct bearing on the formation of the organic millieu, namely the synthesis of porphine-like substances from simple precursors.

EXPERIMENTAL PROCEDURES

It has been known that pyrrole and benzaldehyde when heated

to about 180° C. condense to form α, β, γ, δ -tetraphenyl-porphine (21) (Fig. 1) and α, β, γ, δ -tetraphenylchlorine (22). The same system, with 5 ml. of pyrrole, 10 ml. of benzaldehyde, 10 ml. of pyridine (all freshly redistilled), and 5 g. of zinc acetate, was used in our first experiments (23). The pyridine serves as a solvent. Zinc acetate was found to increase the yield (22, 24). The mixture was placed in a screw-cap Pyrex tube with approximately 2 ml. of air space above the liquid and irradiated with Co^{60} γ-rays for 10 hrs. at the rate of approximately 0.5 megaroentgens per hour. The solution removed from the irradiation vessel, reddish in color, was diluted with chloroform, treated with an equal volume of 6 N hydrochloric acid, and stirred vigorously for 3 hours. The acidic solution was then neutralized with ammonia. The chloroform solution, separated from the aqueous layer, was washed several times with distilled water and chromatographed on activated alumina, as suggested by Priesthoff and Banks (24), with fresh chloroform as the eluent. Three chromatographic separations removed most of the tarry products from the chloroform solution, which was subsequently subjected to chromatography on a Magnesol column. The dark-green band was repacked into a second column, wetted with ether, and eluted with chloroform. The procedure was repeated until a single, clear band was obtained on the column.

In recent experiments (25), a system, consisting of 3 ml. of freshly distilled pyrrole, 6 ml. of benzaldehyde, and 4 ml.

of water, was placed in a 250 ml. beaker, and then irradiated, at a distance of 25 cm with ultraviolet light, using a 100 Watt Hanovia Utility Model Lamp. The radiation transmitted through the filter was 45 per cent at 2500 Å, 90 per cent at 3000 Å and the output of the lamp was 1.5×10^{15} quanta per sec per cm^2 or 5×10^{16} quanta per sec per area of the mixture exposed toward radiation. The doses were measured by chemical actinometry, using an uranyl oxalate actinometer according to Forbes and Heidt (26). After exposure, the reddish-brown solution was diluted with approximately 20 ml. of chloroform and washed with 20 ml. of distilled water. After separation in a separatory funnel, the aqueous layer was discarded, and the chloroform layer was transferred into a beaker charged with approximately 20 g of anhydrous sodium sulfate to remove the excess of water. The sodium sulfate was then filtered off and discarded, while the filtrate was diluted up to 50 ml. volume with chloroform and stored for the quantitative determination of porphines.

The final product separation was accomplished by column chromatography, using activated alumina (F-20) and Florosil (60-100 mesh) as adsorbents and fresh chloroform as eluent. Two chromatographic treatments on alumina and three to four chromatographic separations on Florosil yielded a pure product. The porphines appeared in the eluents and collections of eluent were continued until no Soret band appeared in the effluent.

The same mixture of pyrrole, benzaldehyde and water when placed in the dark without irradiation, also produced porphines.

The isolation and quantitative determination of porphines was done by the same methods used for irradiated mixtures.

If, instead of water, pyridine was used as a solvent in the system, and the mixture was then irradiated for two hours, no porphine-like substances could be detected by previously used methods. Aging of the mixture for ten days produced only traces of porphines.

A typical spectrum of a compound isolated from an irradiated mixture and the reference spectrum of the compound synthesized by the method of Ball, et. al. (22), are presented in Fig. 2. Both spectra are identical with those obtained by Thomas et al. (27), and Dorough et al. (28). For quantitative estimation of the yield, the molar extinction coefficient of 18.7×10^3 at 515 m$_\mu$ maximum was used, as suggested by Thomas and Martell (27). Identity of the compound was further established by the formation of a zinc chelate, according to the procedure of Rothemund and Menotti (29), and Dorough et al. (28). Both spectra, the reference zinc chelate and the chelate of the compound isolated from the irradiated mixture are identical, as shown in Fig. 3.

It is also well known that acid salts of $\alpha, \beta, \gamma, \delta$ - tetraphenylporphines exhibit a characteristic spectra (28, 30). Therefore, part of the benzene solution of the reference compound and part of the benzene solution of compounds isolated from irradiated mixtures were evaporated to dryness and treated with glacial acetic acid. Upon recording the spectra (Fig. 4)

they were found to be identical with those listed in the literature (28, 30).

Although the amount of porphines isolated after γ-irradiation was in the microgram range, this quantity was sufficient to make positive identification.

Fig. 5 and 6 represent the total yields of porphines isolated from the stored mixtures, plotted against time of storage. Each line in these graphs represents different irradiation conditions.

DISCUSSION

In our present study we have shown that porphine-like structures can be synthesized in the presence of oxygen from precursors which were available in the very earliest stage of chemical evolution. Aldehydes were found in products of the action of electrical discharges on the Urey atmosphere in Miller's experiment (4, 5). Pyrroles and pyrrolidines were easily formed from ammonia, acetylene, and other unsaturated hydrocarbons by simple catalysis or under influence of ultra-violet radiation (1). In addition, Lichtin (31) found pyrroles as the major product of the reaction of active nitrogen with 1,3-butadienes.

The results of this study also indicate that the presence of water, or rather the suspension of the organic matter in water, increases the yield of porphine like substances considerably. Oparin (1), and Haldane (3), have already indicated

the possibility of evolution of living matter from the pools
rich in organic matter, rather than on solid surfaces. The
concentration of organic matter could be quite high, if part
of the water solvent evaporated, as the case might be in lakes
and lagoons.

The increase in the yield of porphine-like substances, on
standing, shows that a process of autocatalysis takes place.
This process, postulated by Calvin (19), now can be supported
by experimental evidence.

A closer examination of Fig. 5 and 6 reveals that there is
a difference in the total yield of porphines after standing when
the mixture is originally irradiated with different kinds of
electromagnetic radiation. Initial exposure to the tungsten
lamp and no initial exposure both on standing in semidarkness
seem to have a similar effect. The rate of increase in the
production of porphines is the same within the experimental
error. On the other hand, an initial irradiation with ultra-
violet light has a lower rate of increase of porphine production
under identical conditions of storage. This comparison has
been made on aqueous suspensions of the starting materials
and in the presence of oxygen.

There is a general agreement among many authors that
synthesis of porphyrins is a necessary step for the origin of
life. However, the question of the time of the appearance of
porphyrins is subject to discussion. Gaffron (18), and Calvin
(19), support the ideas that porphyrins were already active in

the very earliest quasi-living organic structures. Miller
and Urey (5), consider this not a necessity and suggest that
porphyrins may have arisen during the evolution of primitive
organisms. Strughold and Ritter (32), consider pre-existing
stores of oxygen, produced by photochemical dissociation of
water, as a prerequisite for the formation and development of
chlorophyll, while the heme types of porphyrins were formed
about 1 to 1.5 billion years later.

Although at the present time it seems to be impossible to
determine with absolute confidence at what stage of chemical
evolution porphine-like substances developed, certain deductions
can be based on the experimental evidence now available. It
can be argued that after the stores of simpler organic compounds
reached their saturation point, in the first stage of chemical
evolution, there was need for a reduction of the energy level
of the source in order to continue chemical evolution to a more
complicated nature. Therefore the photolysis of water and
the formation of an ozone layer cut out short ultraviolet
radiation completely, leaving only a mild energy source of
longer ultraviolet and visible radiation. Considering the
lower rate of production of porphine-like substances on
standing which were irradiated with ultraviolet light, it might
be deduced that the formation of these substances occurred in
an oxidative atmosphere.

In addition evolutionary selection pressure was exerted
for the synthesis of porphine-like substances as soon as the

reductive atmosphere was converted into an oxidative one. Since with the cut off of the short ultraviolet radiation not enough energy could be provided by the long wave ultraviolet and visible radiation for certain chemical synthesis, the appearance of porphines and especially their metal chelates could be considered a necessity. Their aid in chemical transformations such as hydrogen transfer or oxidation is well established.

Also simultaneously with the change of reductive atmosphere to an oxidative one due to the photolysis of water, hydrogen peroxide appeared. If it remained in contact with organic substances, the results would be fatal. Therefore, in order to continue chemical evolution, it was necessary that a mechanism be developed for the destruction of these large amounts of hydrogen peroxide. Calvin (5) has already pointed out that incorporation of ferric iron into a heme- type molecule would increase the catalytic activity of iron for the destruction of hydrogen peroxide by a factor of 10^3, while the addition of certain protein arrangement increases this destruction rate by an additional factor of 10^5.

These last two arguments indicate that with the transition of a reductive atmosphere into an oxidative one, an immediate need was created for the formation of the porphine-like substances.

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OPTICAL DENSITY

SPECTRA IN BENZENE SOLUTION

———— REFERENCE TPP

‑‑‑‑‑ COMPOUND ISOLATED

OPTICAL DENSITY

GLACIAL ACETIC ACID SPECTRA.

—— REFERENCE TPP

–––– COMPOUND ISOLATED

400   500   600   mμ   700

OPTICAL DENSITY

Zn - Chelate SPECTRA in BENZENE

—— Reference Zn-Chelate

–––– Compound isolated

400   450   500   550   600   650 mμ 700